3. (Amended) ~~A~~The plastid transformation and expression vector of claim 1 wherein ~~the immunoglobulin chain~~said immunoglobulin mutimeric chain comprises a light chain.

4. (Amended) ~~A~~The plastid transformation and expression vector of claim 1 wherein ~~the immunoglobulin chain~~said immunoglobulin mutimeric chain comprises both a heavy and a light chain.

5. (Amended) ~~A~~The plastid transformation and expression vector of claim 1 wherein ~~the immunoglobulin chain~~said immunoglobulin mutimeric chain comprises a single-chain variable fragment (scFv).

6. (Amended) ~~A~~The plastid transformation and expression vector of claim 1 wherein ~~the immunoglobulin chain~~said immunoglobulin mutimeric chain comprises a heavy chain constant region fused to an operative ligand.

7. (Amended) ~~A~~The plastid transformation and expression vector of claim 4 wherein ~~the~~said heavy and light chains are separated by a linker comprising an intervening stop codon and a ribosome binding site.

8. (CANCEL) ~~—A~~The plastid transformation and expression vector which comprises an expression cassette comprising as operably linked components, a 5' part plastid spacer sequence, a promoter operative in said plant cell plastids, a selectable marker sequence inclusive of the space sequence, a J chain coding sequence, a transcription termination region functional in said cells and the 3' part of the plastid spacer sequence.

9. (CANCEL)—A vector of claim 8 which comprises a secretory component with the J chain.

10. (CANCEL) A vector of claim 9 in which the secretory component and the J chain are separated by a linker which comprises an intervening stop codon and a ribosome binding site.

11. (Amended) ~~A~~The vector of claim ~~4~~ which ~~comprises~~ further comprises a J chain and a secretory component, thereby producing secretory immunoglobulin A (SigA).

12. (Amended) ~~A~~The plastid transformation and expression vector of claim 1, wherein ~~a~~said 5' part ~~trnA~~of the plastid DNA sequence ~~gene~~ is a plastid flanking sequence, ~~the~~said promoter is a 16S rRNA promoter (Prm) driving ~~the~~said selectable marker sequence~~gene~~, wherein said selectable marker sequence ~~aadA~~ is an aadA gene conferring resistance to spectinomycin, ~~the~~wherein said transcription termination region is a psbA 3' ~~region is a~~ transcription termination region functional in ~~said~~plant cells, and a ~~the trnI gene is the~~said 3' part of the plastid ~~spacer~~DNA sequence is a trnI gene, thereby defining the pLD vector.

13. (Amended) A composition comprising of polypeptide multimer and plant material, wherein said multimer comprises an immunologically active immunoglobulin mutimeric chain, molecule produced from a DNA sequence integrated into the genome of a plant plastid.

14. (Amended) The composition of claim 13 wherein said immunoglobulin mutimeric chain molecule is non-glycosylated.

15. (Amended) The composition of claim 13 wherein ~~the~~said DNA sequence ~~encoding said immunoglobulin molecule~~ comprises at least one sequence encoding a glycosylation signal sequence.

16. (Amended) The composition of claim 14 wherein ~~the~~said DNA sequence ~~encoding said immunoglobulin molecule~~ comprises at least one sequence encoding a glycosylation signal sequence.

17. (Amended) A composition comprising a polypeptide multimer and plant material, wherein said multimer comprises an immunologically active non-glycosyslated immunoglobulin mutimeric chain molecule synthesized in a plant plastid.

18. (Amended) A plant plastid comprising a DNA sequence ~~encoding a polypeptide multimer~~ encoding an immunologically active miltimeric immunoglobulin multimer chain molecule.

19. A plant cell comprising at least one plastid of claim 18.

20. A plant comprising at least one plastid of claim 18.

21. A plant plastid preparation comprising plastids of claim 18.

22. (Amended) A composition prepared from plant plastids of claim 18–, said composition comprising a polypeptide multimer and plant material, wherein said multimer comprises an immunologically active non-glycosylated immunoglobulin mutimeric chain~~prepared from plant plastids of claim 18~~.

23. (Amended) The composition of claim 13 wherein ~~the~~said polypeptide multimer further comprises a J chain.

24. (Amended) The composition of claim 13 wherein ~~the~~said polypeptide multimer further comprises a secretory component.

25. (Amended) The composition of claim 13 wherein ~~the~~said polypeptide multimer further comprises a J chain and a secretory component.

26. (Amended) The composition of claim 17 wherein ~~the~~said polypeptide multimer further comprises a secretory component.

27. (Amended) The composition of claim 17 wherein ~~the~~said polypeptide multimer further comprises a J chain and secretory component.

28. (Amended) A method for introducing DNA encoding immunoglobulin ~~genes~~mutimeric chain coding sequences into a plastid, said method comprising: introducing into a plant cell, ~~with~~ a plastid expression vector adsorbed onto a microprojectile, said plastid expression vector comprising as operably linked components, a DNA sequence containing at least one plastid replication origin functional in a ~~plant~~ plastid, a transcriptional initiation region functional in a ~~said plant~~ plastid, at least one heterologous DNA sequence encoding at least a portion of an immunoglobulin mutimeric chain, at least one DNA sequence encoding a chaperonin and a transcriptional termination region functional ~~inlaid~~in said cells, whereby said heterologous DNA is introduced into a plastid in said plant cell.

29. (Amended) The method of claim 28 wherein ~~the~~ said immunoglobulin mutimeric chain comprises a heavy chain.

30. (Amended) The method of claim 28 wherein ~~the~~ said immunoglobulin mutimeric chain comprises a light chain.

31. (Amended) The method of claim 28 wherein ~~the~~ said immunoglobulin mutimeric chain comprises both a heavy chain and a light chain.

32. (Amended) The method of claim 28 wherein ~~the~~ said immunoglobulin mutimeric chain comprises a single-chain variable fragment (scFv).

33. (Amended) The method of claim 28 wherein ~~the~~ said immunoglobulin mutimeric chain comprises a heavy chain constant region fused to an operative ligand.

34. The method of claim 28 wherein said plastid expression vector further comprises DNA sequences encoding a J chain.

35. The method of claim 28 wherein said plastid expression vector further comprises DNA sequences encoding a secretory component.

36. The method of claim 28 wherein said plastid expression vector further comprises DNA sequences encoding a J chain and a secretory component, thereby producing secretory immunoglobulin (SigA).

37. (Amended) A plastid transformation and expression vector which comprises an expression cassette comprising ~~an~~as operably linked components, a promoter operative in a plastid, a selectable marker sequence, immunoglobulin mutimeric chain coding sequences, a transcription termination region functional in ~~said cells~~a plastid.

38. (Amended) ~~A~~The plastid transformation and expression vector of claim 37 wherein the immunoglobulin chains encoded by said immunoglobulin mutimeric chain coding sequences comprise heavy chains and light chains.

39. (Amended) ~~A~~The plastid transformation and expression vector of claim 38 ~~which~~ wherein said ~~comprises~~immunoglobulin includes covalent bonding between the chains, ~~into~~and said immunoglobulin is immunologically active ~~immunoglobulins~~ in the plastid.

40. (Amended) ~~A~~The plastid transformation and expression vector of claim 39 wherein the heavy and light chains of said encoded immunoglobulin are separated by a linker comprising an intervening stop codon and ribosome binding site.

41. CANCEL A plastid transformation and expression vector which comprises an expression cassette comprising an operably linked components, a promoter operative in plant cell plastids, a selectable marker, a J chain coding sequence, a transcription termination region functional in said cells.

42. CANCEL A vector of claim 41 which comprises a secretory component with the J chain.

43. CANCEL A vector of claim 42 which the secretory component and the J chain are separated by a linker which comprises an intervening stop codon and a ribosome binding site.

44. (Amended) ~~A~~The vector of claim 38 wherein said immunoglobulin mutimeric chain ~~which~~further comprises ~~further~~ a J chain and a secretory component, thereby producing secretory immunoglobulin (SigA).

45. (Amended) ~~A~~The plastid transformation and expression vector of claim 44 ~~which comprises in addition that the~~wherein said light chains are four identical light chains, and ~~the~~said heavy chains are four chains.

46. (Amended) ~~A~~The plastid transformation and expression vector of claim 38 wherein ~~the~~said promoter is a 16S rRNA promoter (Prrn), ~~driving the~~said selectable marker sequence encodes the gene aadA, conferring resistance to spectinomycin, and ~~the~~said ~~psbA 3' region is a~~ transcription termination region functional in ~~said cells~~a plastid is a psbA 3' region, thereby defining the pZS vector.

47. (Amended) ~~The~~A stably transformed plant which has been transformed by the vector of any one of claims 37 - 46.

48. The progeny, including but not limited to seeds, of the stably transformed plant of claim 47.

49. The plant of either one of claim 47 or claim 48, wherein the plant is tobacco.

50. (Amended) A universal plastid transformation and expression vector which comprises an expression cassette ~~comprising~~ as operably linked components, a 5' part of ~~the~~a plastid spacer sequence, a promoter operative in ~~said plant cell~~ plastids, a selectable marker sequence ~~marker~~, at least one DNA sequence encoding at least a portion of an immunioglobulin mutimeric chain, at least one DNA sequence encoding a chaperonin, a transcription termination region functional in ~~said cells~~plastids and ~~the~~a 3' part of ~~the~~a plastid spacer sequence, and flanking each side of the expression cassette, flanking DNA sequences which are homologous to ~~a~~ DNA sequences inclusive of ~~a~~ spacer sequences conserved in the plastid genome of different plant species, whereby stable integration of said immunoglobulin mutimeric chain~~the heterologous~~ coding DNA sequence into ~~the~~a plastid genome of ~~the~~a target plant is facilitated through homologous recombination of ~~the~~said flanking DNA sequences with ~~the~~ homologous sequences in the target plastid genome.

REMARKS

The application has been restricted under and 35 U.S.C. § 121 and § 372 requiring the election of one invention from among the Groups I-VIII. Applicants respectfully traverse this Restriction Requirement, but in order to be in full compliance within the election of the restriction requirements the Applicants hereby elect to prosecute the invention contained in Group I and corresponding to claims 1-7 and 28-33, which is drawn to a plastid transformation vector comprising a 5' plastid DNA sequence, a plastid promoter, a selectable marker sequence, a sequence encoding a single-chain variable fragment, a transcription termination region and a 3' plastid DNA sequence, and a method for transforming plants with the vector.

Applicants respectfully submit that the Restriction Requirement should now be removed as a result of the Applicants amendments which are in accordance with the Examiner's Interview Summary provided to the Applicants on October 17, 2002. Specifically, the Applicants have amended the claims to ensure that the technical features shared by Groups I-VIII relate to a vector encoding a chaperonin and a mutimeric chain immunoglobulin. As a result of these amendments, Mayfield, *et al.* (WO 98/31823, cited in the PCT Search Report) no longer discloses the Applicants' shared technical feature.

Applicants respectfully submit that Mayfield *et al.* failed to show an enabled expression of a dimeric IgA. Specifically, Mayfield *et al.* is not enabled because the assembly of multimeric antibodies requires the presence of a chaperonin. Mayfield *et al.* never suggested or appreciated the use of chaperonins as necessary for the expression of multimeric antibodies. The Examiner's attention is invited to the following passage of the Applicants' specification:

> In mammalian plasma cells the immunoglobulin light and heavy chains, encoded by nuclear genes, are synthesized as precursor proteins containing an amino-terminal signal peptide that guides the chains into the lumen of the endoplasmic reticulum (ER). The signal peptide is cleaved off in the ER and stress proteins such as BiP/GRP78 and GRP94, which function as chaperonins, bind to unassembled light and heavy chains and direct their folding and assembly (Gething and Sambrook., 1992; Melnick *et al.*, 1992). Disulfide bond formation is catalyzed by protein disulfide isomerase and N-linked glycans are attached in the ER and further processed in the Golgi, before the antibody is secreted from the cell.
>
> On the other hand, it has been known for some time that disulfide bonds exist both within (Ferri *et al.*, 1978) and between some

plastid proteins (Ranty *et al.*, 1991; Schreuder *et al.*, 1993; Drescher *et al.*, 1998). Both nuclear and plastid encoded proteins are activated by disulfide bond oxidation/reduction cycles using the plastid thioredoxin system (Ruelland and Miginiac-Maslow, 1999) or plastid protein disulfide isomerase (Kim and Mayfield, 1997). Chaperonin molecules of the HSP70 and HSP60 families, including the rubisco binding protein, have also been reported in plastids (Roy, 1989; Vierling, 1991). These molecules function in the folding and assembly of eukaryotic (nuclear) and prokaryotic (plastid) proteins. We hypothesized that they would be able to assist in the proper assembly of immunoglobulin chains in plastids.

These are examples of protein complexes in the plastid in which all the subunits are native tot he plant, the ribosome being an example. However, the expression and assembly in transformed plastids of heterologous proteins into multi-protein complexes has not been reported until the present invention. There is a single example in the literature of an inter-chain disulfide bond in plant plastids, and that is between neighboring large subunits of the enzyme ribulose-1, 5-biphosphase carboxylase/oxygenase (Ranty et al., 1991). The expression and assembly in transformed plastids of functional proteins consisting of different protein chains, including disulfide bonds between different subunits, as represented by expression and assembly of a mammalian antibody has never been demonstrated until the present invention.

The Applicants have demonstrated that chaperonins are necessary in order to correctly assemble multimetric antibodies in the plastids. As a result, the expression in assembly of multimetric mammalian antibodies had never been demonstrated until the present invention.

Support for the Applicants' amendments can be found on page 5, lines 1-10, and page 6, lines 20-22, page 10 lines 7-14, and Example 1 of the specification. No new natter has been added as result of the amendment.

Respectfully submitted,

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